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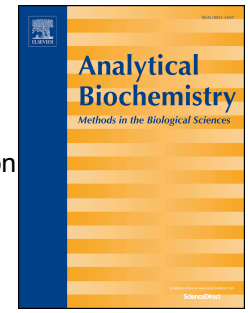

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Evaluation of diffusion coefficients by means of an approximate steady-state condition in sedimentation velocity distributions

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ABSTRACT

This investigation examines the feasiblity of manipulating the rotor speed in sedimentation velocity experiments to generate spontaneously an approximate steady-state condition where the extent of diffusional spreading is matched exactly by the boundary sharpening arising from negative *s*-*c* dependence. Simulated sedimentation velocity distributions based on the sedimentation characteristics for a purified mucin preparation are used to illustrate a simple procedure for determining the diffusion coefficient from such steady-state distributions in situations where the concentration dependence of the sedimentation coefficient, $s = s^0/(1 + Kc)$, has been quantified in terms of the limiting sedimentation coefficient as $c \rightarrow 0$ (s^0) and the concentration coefficient (K). Those simulations have established that spontaneous generation of the approximate steady state could well be a feature of sedimentation velocity distributions for many unstructured polymer systems because the requirement that $Kc_o\omega^2 s^0/D$ be between 46 and 183 cm^{-2} is not unduly restrictive. Although spontaneous generation of the approximate steady state is also a theoretical prediction for structured macromolecular solutes exhibiting linear concentration dependence of the sedimentation coefficient, $s = s^0(1 - kc)$, the required value of k is far too large for any practical advantage to be taken of this approach with globular proteins.

Keywords:

Diffusion coefficient

Sedimentation velocity

Ultracentrifugation

In sedimentation velocity experiments on a noninteracting macromolecular solute the spreading of the migrating boundary by diffusion is countered to some extent by negative concentration dependence of the sedimentation coefficient (s). Allowance therefore needs to be made for this boundary sharpening in order to obtain a meaningful estimate of the diffusion coefficient (D). Advantage was taken long ago [1–4] of an approximate analytical solution [5,6] of the basic sedimentation equation [7] under constraints of linear s–c dependence but concentration-independent diffusion to evaluate D for globular proteins.

An alternative analytical approach entails manipulation of the sedimentation conditions to achieve an approximate steady-state condition in which the extent of diffusional spreading is matched exactly by the boundary sharpening arising from negative s-c dependence [8,9]. Unfortunately, the technical complexities associated with the suggested procedure for achieving that steady-state condition in the sedimentation of globular proteins have led to virtual disregard of the approach for evaluating diffusion coefficients. However, subsequent numerical solution of the Lamm equation under the same constraints [10] has revealed that the steady-state condition should arise in conventional sedimentation velocity experiments on systems exhibiting a sufficiently large s-c dependence. This time-invariant shape for sedimentation velocity distributions has also been observed as the limit of boundary spreading analysis based on the general solution of the Lamm equation by the SEDFIT program [11].

In this investigation we employ that ~~the~~ more recent SEDFIT computer program [12,13] for numerical solution of the Lamm equation to provide additional confirmation of that earlier observation [10] as well as to show that the steady-state condition would have been attained in normal sedimentation velocity distributions for the mucin preparation examined by Creeth [9] We also shed further light on the range of solute sedimentation characteristics (s^0, D and Kc_o, the

product of initial solute concentration and the concentration coefficient) compatible with spontaneous steady-state generation.

Theory

In terms of solute flow for a single, non-interacting solute in solvent the Lamm equation may be written as

$$J(r) = \omega^2 rsc - D(dc/dr) \quad (1)$$

where $J(r)$ is the flow of solute at radial distance r from the centre of rotation of a rotor spun with angular velocity ω. Being a function of concentration, s refers to the sedimentation coefficient pertaining to the solute concentration (c) at radial distance r. The flow of solute mediated by the centrifugal field is opposed by diffusional flow, which is depicted in Eq. (1) as the product of the diffusion coefficient D (assumed constant) and the solute concentration gradient (dc/dr) at radial distance r. In that regard the absence of a concentration gradient in the plateau region means that the flow of solute there is given by

$$[J(r)]_p = \omega^2 r s_p c \quad (2)$$

where s_p is the invariant sedimentation coefficient describing solute migration throughout the plateau region because of the constant concentration c_p. The establishment of a steady state in the boundary region is thus conditional upon identity of the flow defined by Eq. (1) with that, $[J(r)]_p$, in the plateau region, a situation described by the relationship

$$\omega^2 rc(s - s_p) - D(dc/dr) = 0 \quad (3)$$

or, upon rearrangement

$$\frac{1}{rc}\frac{dc}{dr} = \frac{\omega^2(s - s_p)}{D} \tag{4}$$

For relatively unstructured macromolecular solutes the concentration dependence of the sedimentation coefficient is described adequately by the relationship

$$s = s^0/(1 + Kc_p) \tag{5}$$

where s^0 is the limiting sedimentation coefficient as $c_p \rightarrow 0$; and where the upper case notation is used for the concentration coefficient in order to distinguish it from the corresponding parameter for globular proteins, which exhibit a linear concentration dependence of sedimentation coefficient, $s = s^0(1 - kc_p)$. From Eq. (5) the difference between the magnitudes of the sedimentation coefficient for concentrations c and the plateau concentration c_p may be written

$$s - s_p = s^0 K \frac{(c - c_p)}{(1 + Kc)(1 + Kc_p)} \tag{6}$$

which, on combination with Eq. (4) and integration, leads to the expression [9,10]

$$Y = \ln (c/c_o) - (1 + Kc_p) \ln [(c_p - c)/c_o] = \frac{\omega^2 s^0}{2D} \frac{Kc_p}{1 + Kc_p} (r^2 - r_b^2) \quad (7)$$

where r_b denotes the boundary position. For these systems the location of r_b is not straightforward because of boundary asymmetry and hence the need to determine the boundary position as the square root of the second moment of the boundary [14,15]. This reference radial position has also been described [10] as the radius at which $\ln c = (1 + Kc_p) \ln (c_p - c)$. Fortunately, the determination of r_b is not crucial to the analysis in that an estimate of D is already available [10] from the slope of the dependence of $[(1 + Kc_p) \ln (c_p - c) - \ln c]$ upon r^2, provided that values for s^0 and K have been pre-determined. For comparisons of boundary spreading as a function of time (or distance migrated) it therefore suffices to regard r_b as the radial distance at which $Y = 0$.

Practical Considerations

The application of this approach for the determination of D from the extent of boundary spreading in a sedimentation velocity experiment is, of course, dependent upon the conformity of solute distributions with the steady-state criterion. In that regard the previous test for compliance [9] was based on availability of the distribution in schlieren format (the radial dependence of dc/dr) – the usual optical record of sedimentation velocity distributions at that time. We now require a criterion for the radial dependence of concentration (or absorbance), the form in which sedimentation velocity distributions are currently recorded – a requirement that is verified in the present simulative study by return of the input value of the diffusion coefficient.

Feasibility of spontaneous generation of steady-state distributions for a mucin

At present the only evidence for the possibility of steady-state distributions being generated spontaneously in sedimentation velocity experiments resides in Fig. 15 of the computer simulation study by Dishon and coworkers [10], which demonstrates conformity of distributions with Eq. (7) for two situations: one in which $\omega^2 s^0/D = 23.5$ cm^{-2} and $Kc_o = 5$ (Fig. 15A therein); and the other in which $\omega^2 s^0/D = 117$ cm^{-2} and $Kc_o = 1$ (Fig. 15B therein). Although the former possible situation is unlikely to be encountered experimentally because of its requirement for an extremely large s–c dependence, the sedimentation characteristics ($s^0 = 11.1$ S, $K = 0.12$ L/g, $D = 1.5 \times 10^{-7}$ cm^2 s^{-1}) of the B-specific blood group substance [15] studied by Creeth [9] conform fairly closely with the second possibility in that $\omega^2 s^0/D = 130$ cm^{-2} and $Kc_o = 0.9$ for a 7.5 g/L solution of the mucin subjected to centrifugation at 40,000 rpm.

To provide sedimentation velocity distributions for testing the above theoretical predictions, concentration profiles were generated by the SEDFIT program [12,13], a moving-hat adaptation [12,17] of the Claverie finite element approach [18] to solving the differential equations numerically. Simulated distributions were recorded at 5-minute intervals for a solution of solute subjected to centrifugation at selected rotor speeds: a random error of 0.02 fringes was superimposed on the simulated distributions to replicate the experimental situation [19].

Distributions at 5-minute intervals were generated for a 7.5 g/L solution of mucin subjected to centrifugation at 20,000–50,000 rpm. Representative concentration patterns are shown in Fig. 1A, which presents distributions generated after 25–200 min of simulated centrifugation at 40,000 rpm in experiments with $c_o = 7.5$ g/L for $6.2 \le r \le 7.2$ cm as the initial boundary condition. A striking feature of these patterns is the similarity of shape for all distributions as the boundary

migrated the entire cell length – a characteristic that contrasts with the time-dependent increase in diffusional spreading that is usually encountered in sedimentation velocity studies on smaller solutes at higher rotor speeds. Analyses of these distributions in accordance with Eq. (7) exhibited the predicted linear dependencies of Y upon $(r^2 - r_b^2)$ across the entire boundary ($0.02 < c/c_p < 0.98$), the progressive decrease in the slopes of which reflected the decline in plateau concentration (Fig. 1A) because of radial dilution in a sector-shaped cell [15]. The degree of conformity with attainment of the steady-state condition is thus demonstrated more readily by rearranging Eq. (7) in the form

$$[(1 + Kc_p)/(Kc_p)]Y = [1/(2D)]\omega^2 s^0 (r^2 - r_b^2) \qquad (8)$$

which allows determination of $1/(2D)$ from a common linear dependence of $[(1 + Kc_p)/(Kc_p)]Y$ upon $\omega^2 s^0$ for all concentration distributions. This feature is confirmed for the current system after simulated centrifugation for 25 (◆), 75 (◇), 125 (▲) and 200 (△) minutes in Fig. 1B, where the solid line is the predicted dependence with slope $\omega^2 s^0/(2D)$.

The results presented in Figs. 1A and 1B have thus demonstrated that a steady-state sedimentation velocity condition would have arisen spontaneously in ultracentrifuge studies of the mucin preparation at 40,000 rpm, thereby avoiding the need to adopt the far more tedious sedimentation velocity procedure used originally [9]. In that regard this demonstration merely confirms an earlier finding [10] because the rotor speed and initial solute concentration were chosen to yield the value of 117 cm^{-2} for $Kc_o\omega^2 s^0/(2D)$ that Dishon and coworkers [10] had shown to be commensurate with spontaneous steady-state generation. Nevertheless, it draws attention to the potential of the simplified steady-state approach in sedimentation velocity studies of mucins and proteoglycans, which are characterized by much smaller diffusion coefficients and greater *s–c*

dependence than those exhibited by globular proteins – a consequence of their lack of the higher-order structure responsible for the compactness the latter solutes.

Further examination of the range of conditions amenable to steady-state attainment

As noted in the previous section, the decision to examine the mucin system at 40,000 rpm reflected the conformity of its sedimentation characteristics with those of the system depicted in Fig. 15B of Dishon and coworkers [10]. Furthermore, the same value of $Kc_o\omega^2 s^0/D$ (117 cm^{-2}) also applies to the other situation ($\omega^2 s^0/D$ =23.5, Kc_o = 5) for which evidence of steady-state attainment was provided (Fig. 15A of [10]). Simulations of sedimentation velocity distributions for the same mucin solution (c_o = 7.5 mg/mL) at a range of rotor speeds (20,000–50,000 rpm) were therefore undertaken to determine the range of $Kc_o\omega^2 s^0/D$ values commensurate with spontaneous steady-state attainment, and hence to shed further light on the potential utility of this approach to physicochemical characterization of unstructured macromolecular solutes.

Repetition of the SEDFIT simulation protocol for the same mucin solution (c_o = 7.5 mg/mL) at rotor speeds of 50,000 and 25,000 rpm yielded the sedimentation velocity distributions presented in Figs. 2A and 2B respectively. Despite differences in the extent of boundary spreading, both series of patterns share with their counterparts in Fig. 1A a lack of any time-dependence of that spreading. Confirmation that these two sets of distributions also reflect spontaneous attainment of the approximate steady state is provided by their analysis in terms of Eq. (8), which is summarized in Fig. 3A to establish that results from distributions for a given rotor speed are characterized by the same linear dependence upon r^2 – a slope that increases with increasing rotor speed: the additional data set in Fig. 3A reflects the results of simulations at 30,000 rpm. The theoretical requirement for these slopes to exhibit a linear dependence upon the product of s^0 and the square of angular velocity

is demonstrated in Fig. 3B, which also includes the corresponding analyses of simulated sedimentation velocity distributions at intermediate rotor speeds (32,000, 34,000, 36,000, 40,000, 44,000, 46,000 and 48,000 rpm). Furthermore that linear dependence of those slopes coincides with the solid line in Fig. 3B, which is the corresponding theoretical dependence with a slope of 1/(2D), for the present system under conditions reflecting the attainment of the approximate steady state [Eq. (8)].

The present considerations have thus established that spontaneous generation of the approximate steady state should be a feature of sedimentation velocity distributions for unstructured polymer systems with values of $Kc_o\omega^2s^0$/D between 46 and 183 cm^{-2}. Although extension of this range beyond the upper limit is certainly possible theoretically, use of the steady-state condition for determination of the diffusion coefficient then becomes dependent upon other considerations. In the event that the extent of boundary sharpening were to exceed diffusional spreading, the spontaneously generated steady state distribution would comprise a hypersharp boundary in which c increases from zero to c_p in stepwise fashion at radial distance $r = r_m\exp(\omega^2st)$ [20]. Furthermore, there is a distinct possibility that instrumental limitations in Rayleigh fringe resolution may preclude adoption of the present analysis for systems approaching that limiting steady-state situation. Indeed, from a practical viewpoint that experimental limit may already have been exceeded in that the above analysis of simulated distributions for the mucin preparation at 50,000 rpm (Fig. 2A) would be conditional upon an instrumental resolving power of 2,300 fringes/cm.

The above value of 46 cm^{-2} almost certainly overestimates the lower limit of $Kc_o\omega^2s^0/D$ for spontaneous steady-state attainment, which is, however, greater than 30 cm^{-2}. Spontaneous generation of the approximate steady state certainly was certainly not observed in simulated sedimentation velocity distributions at 20,000 rpm ($Kc_o\omega^2s^0/D$ = 29) – a factor evident from their

analysis (Fig. 4) according to Eq. (8): the curvilinearity and non-superimposition of results for distributions obtained after 200 and 600 minutes of simulated centrifugation are clearly incompatible with the theoretical requirements for steady-state attainment. Nevertheless, the present demonstration of spontaneous steady-state attainment in sedimentation velocity experiments on systems for which $46 \le Kc_o\omega^2 s^0/D \le 183$ cm^{-2} has already sufficed to signify the potential relevance of this relatively simple analysis to the physicochemical characterization of large unstructured polymers such as polysaccharides and mucins. Some potential examples are listed in Table 1, which additionally includes fibrinogen – a structured but highly asymmetric protein that also exhibits *s*–*c* dependence described by Eq. (5).

Feasibility of spontaneously generated steady-state distributions for a globular protein

For globular proteins the concentration dependence of the sedimentation coefficient is described adequately by the relationship

$$s = s^0(1 - kc_p) \tag{9}$$

where s^0 is the limiting sedimentation coefficient as $c_p \to 0$. However, the combination of a smaller *s*–*c* coefficient (k) and a larger diffusion coefficient for a compact particle diminishes the likelihood of obtaining a sufficiently large value of $kc_o\omega^2 s^0/D$ for spontaneous generation of a steady-state concentration distribution. By analogous reasoning to that adopted for systems exhibiting linear concentration dependence of $1/s^0$ the counterpart of Eq. 7 becomes [9,10]

$$\ln[(c_p - c)/c] = -\frac{c_p\omega^2 s^0 k}{2D}(r^2 - r_b^2) \tag{10}$$

where r_b denotes the boundary position (radial distance at which $c = c_p/2$) for the symmetrical boundary obtained with systems exhibiting linear *s*–*c* dependence. Subject to the pre-determination of values for s^0 and *k*, the linear dependence of $(1/c_p)\ln[(c_p - c)/c]$ upon $(r^2 - r_b^2)$ provides a means of evaluating *D* – the only parameter of unknown magnitude in Eq. 10 – subject to demonstration that the steady-state condition has been attained.

For these systems exhibiting linear *s*–*c* dependence an alternative criterion for steady-state attainment in a *c*–*r* distribution is obtained by rewriting Eq. (10) as

$$\frac{1}{c_p} \ln [(c_p - c)/c] = - \frac{\omega^2 s^0 k}{2D} (r^2 - r_b^2) \qquad (11)$$

which requires coincidence of the dependencies of $(1/c_p) \ln [(c_p - c)/c]$ upon $(r^2 - r_b^2)$ for all distributions in which a plateau region is preserved. Analysis of several distributions from the same sedimentation velocity run may thus suffice to establish their conformity with the steady-state condition. Alternatively, repetition of the sedimentation velocity experiment with a different loading concentration or rotor speed provides a more rigorous test of compliance because of a greater range in c_p that can be used in Eq. (11).

For globular proteins the magnitude of $kc_p\omega^2 s^0/D$ is too small for the steady-state condition to be established, and hence the inapplicability of the suggested procedure for *D* determination should become evident from the plot of results according to Eq, (11). This aspect of the procedure is illustrated in Fig. 5, which summarizes such analyses (Fig. 5B) of distributions (Fig. 5A) obtained by the SEDFIT program [12,13] for a single-solute system with the ultracentrifugal characteristics of a 10 g/L solution (c_o) of horse γ-globulin (s^0 = 7.38 S, k = 0.00785 L/g and $D = 4.8 \times 10^{-7}$ cm^2 s^{-1}) subjected to centrifugation at 56,100 rpm – the highest rotor speed employed in the original

detailed study of this protein by the more complicated but more generally applicable steady-state approach [9] Although the plots are linear, the systematic decrease in slope with decreasing c_p signifies that the extent of boundary sharpening arising from the negative s–c dependence only partially counters diffusional spreading of the boundary. Analytical determination of the diffusion coefficient from concentration distributions such as those shown in Fig. 5A would thus require their consideration in terms of the approximate solution of the Lamm equation devised by Fujita [5,6].

Failure to attain the spontaneous steady-state condition for horse γ-globulin can be rationalized in terms of the small magnitude of $kc_o\omega^2 s^0/D$, namely 4 cm^{-2}, that applies to this 150 kDa protein at 56,100 rpm. Furthermore, a similar situation would also apply to rabbit muscle pyruvate kinase, a 240 kDa globular protein for which s^0 = 10.0 S, k = 0.0086 L/g and $D = 4.0 \times 10^{-7}$ cm^2 s^{-1} [24], because the 60% increase in size merely raises the value of $kc_o\omega^2 s^0/D$ to 5 cm^{-2} for a 10 g/L solution subjected to centrifugation at maximum rotor speed (60,000 rpm).

Discussion

The above examples have illustrated the feasibility of steady-state attainment in conventional sedimentation velocity studies on macromolecular solutes exhibiting linear concentration dependence of 1/s [Eq. (5)] over a relatively wide range of $Kc_o\omega^2 s^0/D$ values; and hence signified the likelihood of its occurrence in many ultracentrifugal studies of unstructured macromolecular solutes. Furthermore, as implied by the observations of Dishon and coworkers [10] determination of the diffusion coefficient from these spontaneously generated steady-state sedimentation velocity distributions is sufficiently simple for the approach to attract far more attention than its predecessor [9], which entailed initial centrifugation at 47,660 rpm for 20 min to clear the solute boundary from the meniscus, after which the speed was decreased to 20,410 rpm for 300 min. The rotor speed was then increased to 24,630 rpm and maintained at that value for a

further 320 min to obtain five distributions reflecting steady-state attainment. That protocol was, of course, designed [9] in the belief that attainment of the steady-state condition would require a lesser centrifugal force than that commensurate with conventional velocity sedimentation – a belief seemingly confirmed by the rotor speed of 24,630 rpm used to generate the steady-state distributions.

An important point to emerge from the present investigation is the demonstration that steady-state sedimentation velocity distributions may be attainable for a given macromolecular solute system over a relatively wide range of rotor speeds. There is therefore justification for optimism that distributions obtained by the standard sedimentation velocity protocol will yield patterns that are amenable to steady-state analysis. Only in the event that a rotor speed lower than that commensurate with spontaneous generation of a boundary between solvent and solution plateaux is required to achieve the approximate steady state condition should resort need to be made to the technically more demanding procedure described in the pioneering investigation [9] that raised the possibility of obtaining s^0/D by this relatively simple analysis.

An obvious limitation of this relatively simple analysis of steady-state sedimentation velocity distributions for diffusion coefficient determination is its reliance upon homogeneity of the macromolecular solute – an assumption that would obviously require close scrutiny in any experimental situation because of the polydispersity exhibited by many unstructured polymers. Of additional concern is the assumed concentration-independence of the translational diffusion coefficient, an approximation that has been rendered difficult to verify by the essential demise of procedures for measuring D under the constraints of constant temperature and solvent chemical potential [25]. Although attention to these two aspects is clearly required in future studies attempting to take advantage of the suggested procedure, the present theoretical considerations have

at least provided the necessary background and hence justification for further exploration of the method.

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Table 1

Unstructured or highly asymmetric polymeric solutes that seemingly exhibit the requirement for spontaneous steady-state attainment in sedimentation velocity experiments

Solute	s^0 (S)	K (L/g)	$10^7 D$ ($cm^2 s^{-1}$)	Ref.	Rotor speed (rpm)	$\omega^2 s^0 K c_0/D$ [a] (cm^{-2})
Mucin[b]	11.1	0.12	1.5	[9]	40,000	78
Mucin[c]	3.9	0.083	0.93	[21]	50,000	87
Xylinan	9.5	0.27	0.30	[22]	20,000	188
Fibrinogen	8.0	0.045	2.0	[23]	60,000	36

[a] Value for a 5 g/L solution of macromolecular solute.

[b] B-specific blood group substance discussed above.

[c] Tryptic fragment of reduced cervical pregnancy mucin.

LEGENDS TO FIGURES

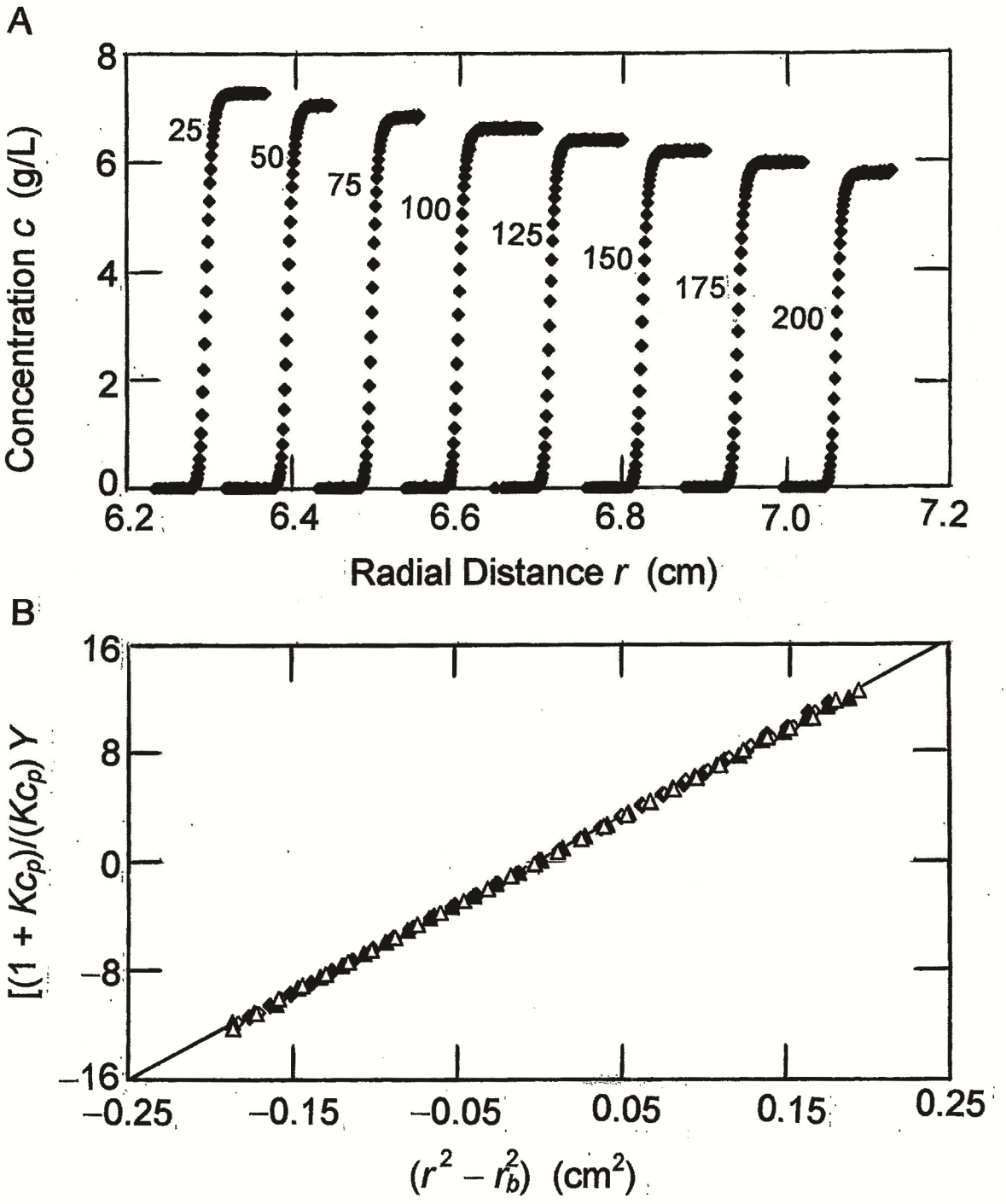
Fig. 1. Simulated migration of a 7.5 g/L solution of a mucin preparation (s^0=11.1 S, K = 0.12 L/g, $D = 1.5 \times 10^{-7}$ cm^2/s) subjected to centrifugation at 40,000 rpm in an experiment with the air–liquid meniscus located at r = 6.2 cm. (A) Concentration distributions obtained after the indicated time periods (minutes) of centrifugation. (B) Use of Eq. (8) to analyze the distributions after migration for 25 (◆), 75 (◇), 125 (▲) and 200 (△) minutes.

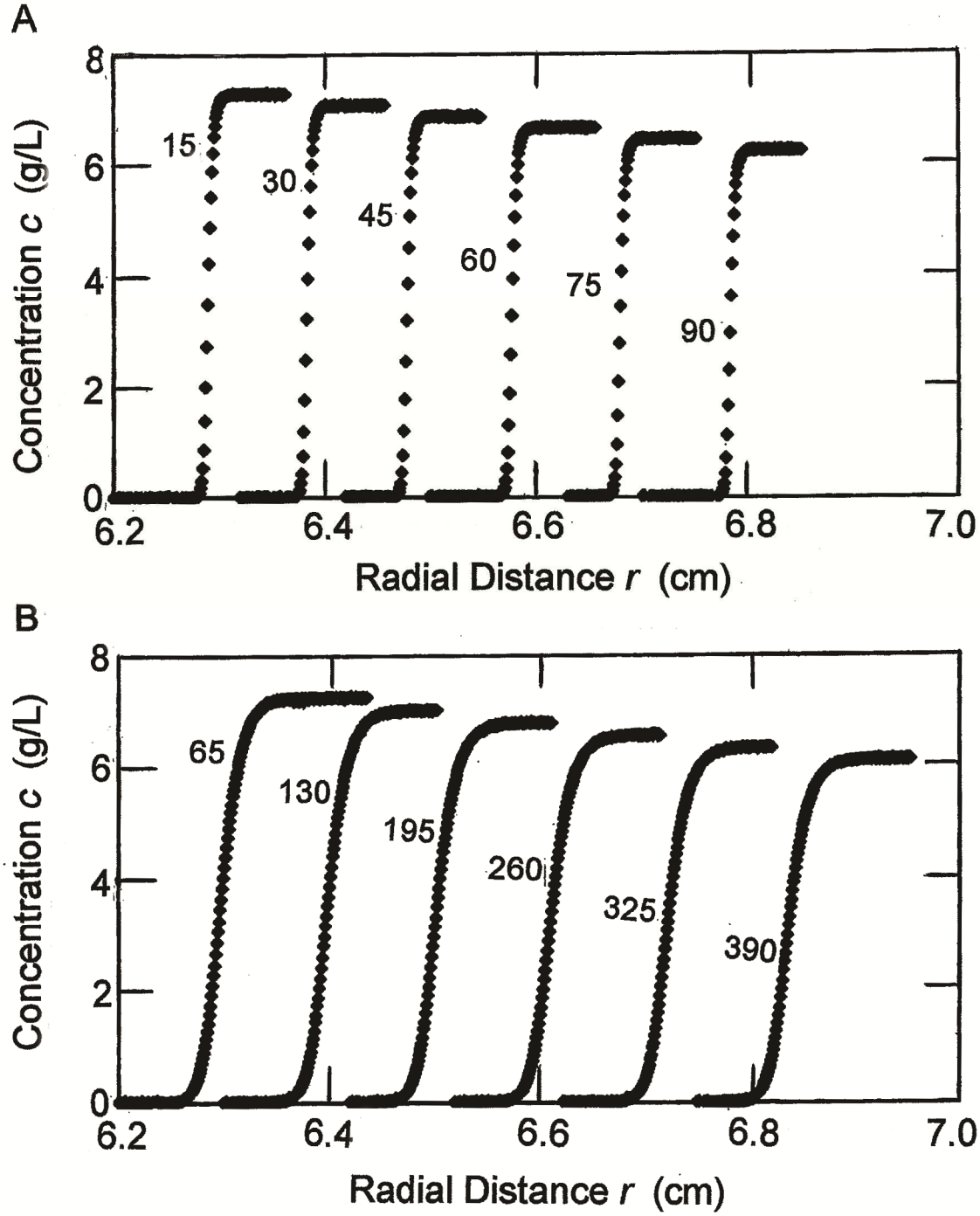
Fig. 2. Extension of the range of rotor speeds commensurate with spontaneous generation of the approximate steady state for the mucin preparation considered in Fig. 1. (A) Concentration distributions resulting from simulated centrifugation at 50,000 rpm for the indicated periods (minutes). (B) Sedimentation velocity distributions obtained by repeating the simulation at 30,000 rpm.

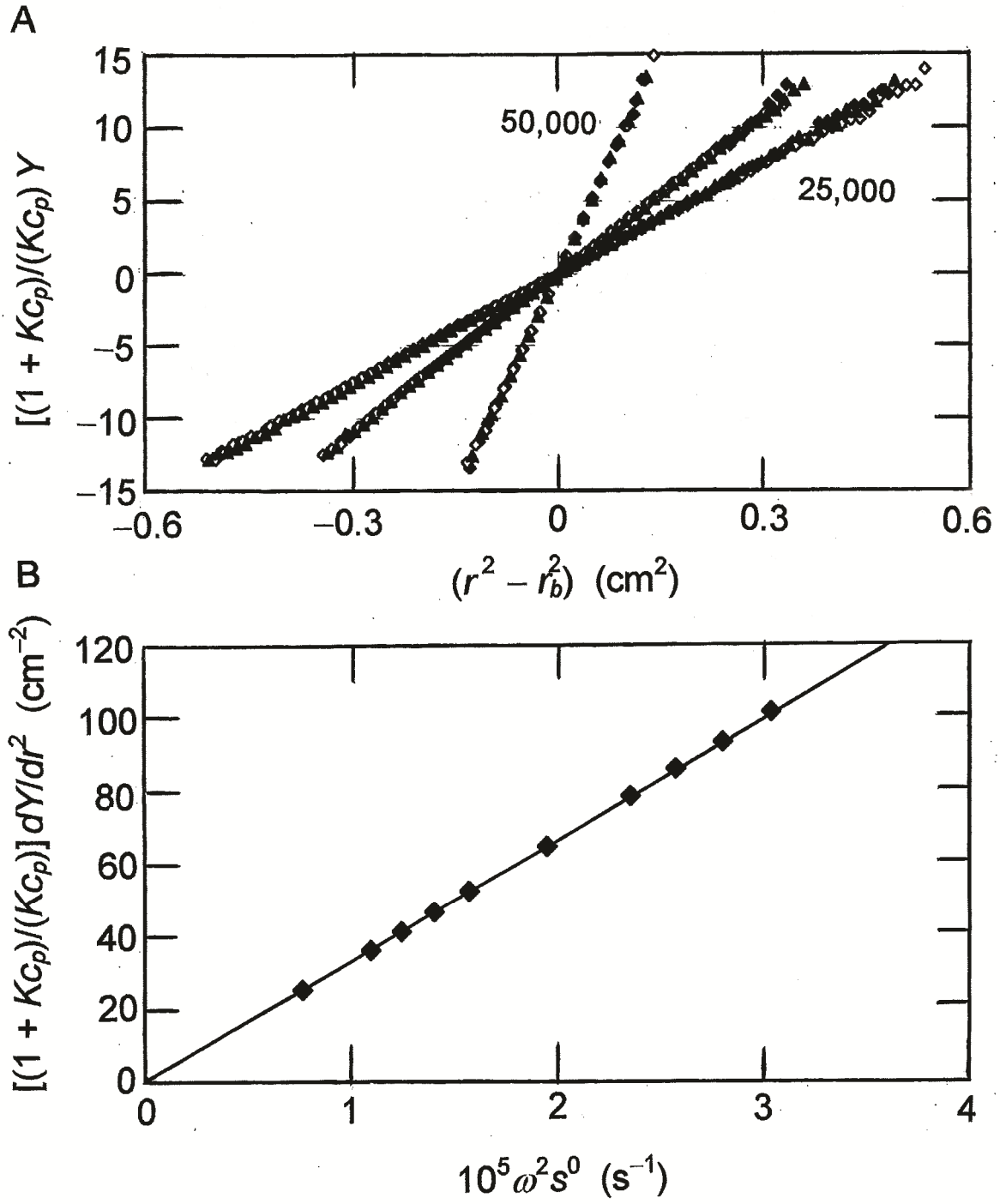
Fig. 3. Confirmation of spontaneous steady-state attainment in simulated sedimentation velocity experiments on the mucin preparation at a range of rotor speeds. (A) Demonstration of the essential independence of boundary spreading upon distance migrated by its analysis in terms of Eq.(8). (B) Consequent analysis to establish that the slopes of such plots (Fig. 4A) for those and other intermediate rotor speeds comply with values commensurate with steady-state attainment: the solid line signifies the theoretical dependence predicted by Eq. (8).

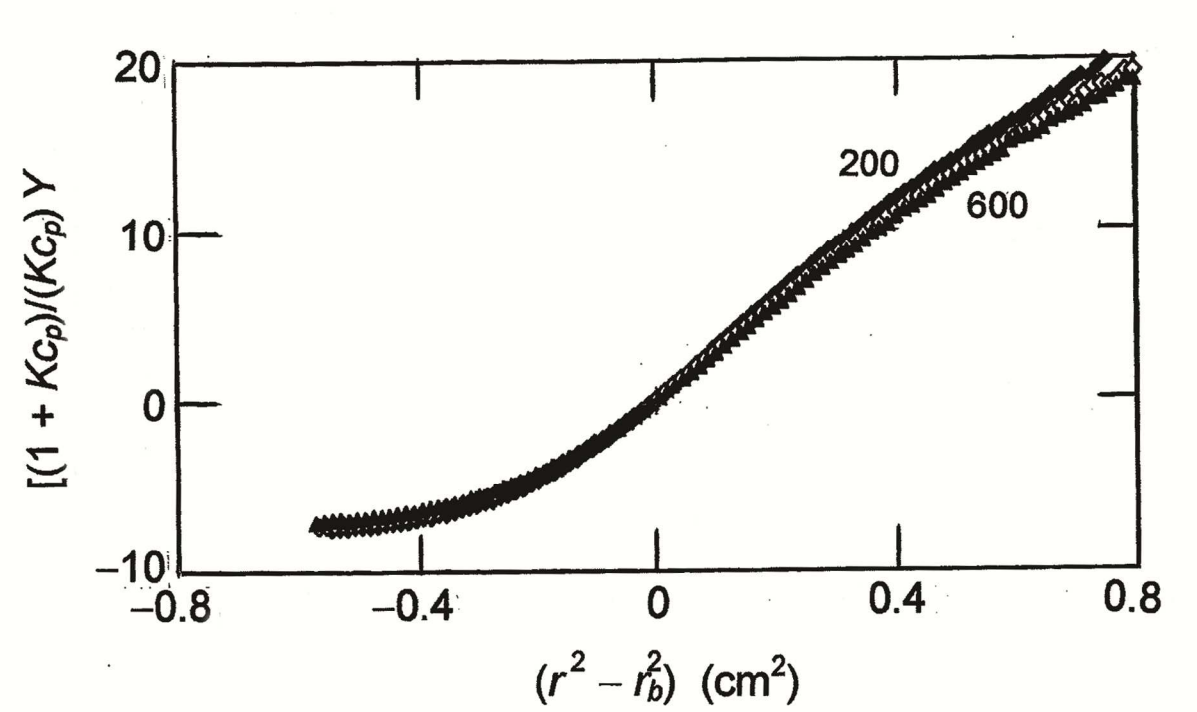
Fig. 4. Nonconformity of simulated sedimentation distributions for the mucin preparation subjected to centrifugation at 20,000 rpm with the time-independent, linear relationship predicted by Eq. (8) for steady-state attainment.

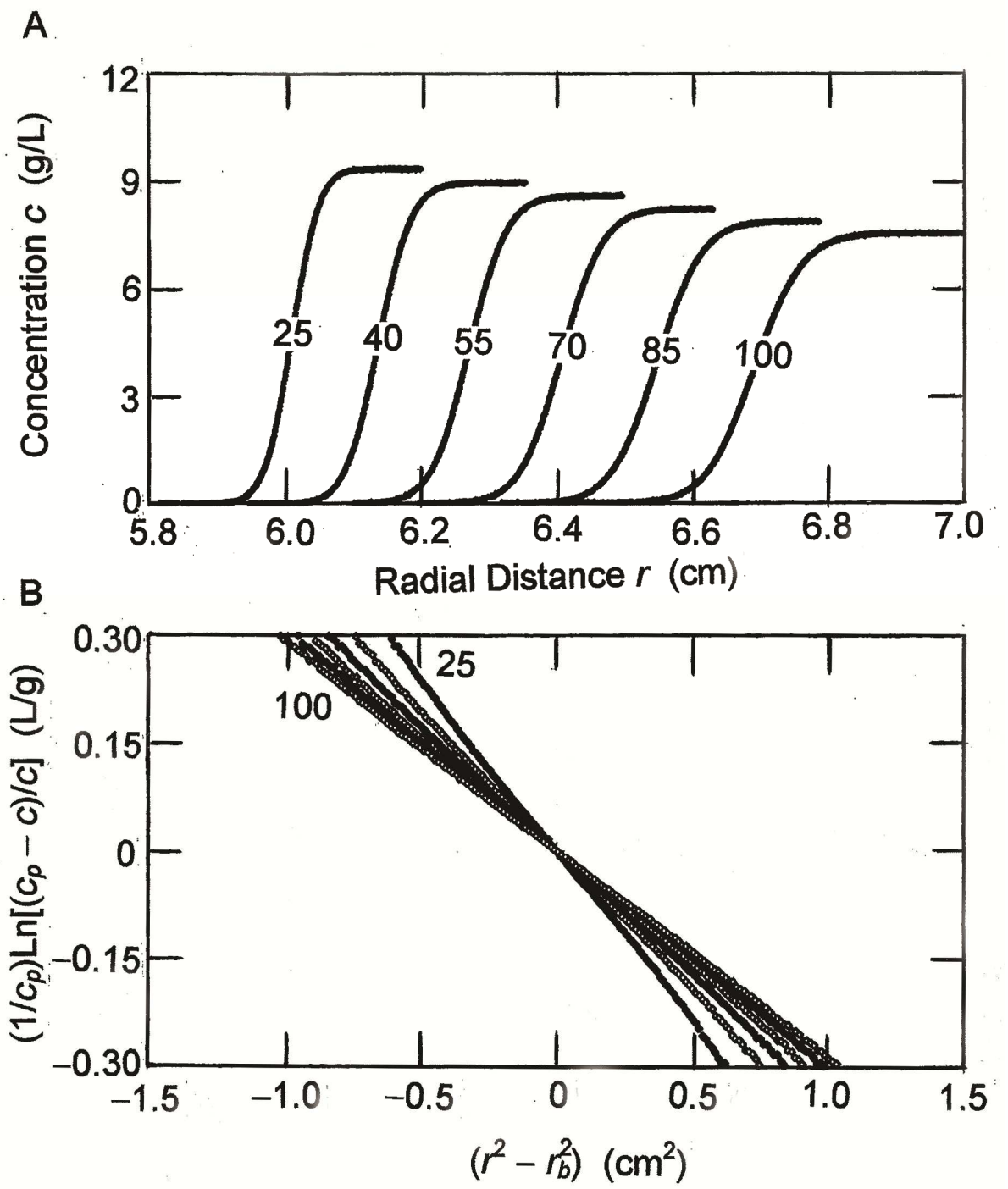
Fig. 5. Demonstrated non-compliance of simulated sedimentation velocity distributions with steady-state attainment for a 10 g/L solution of horse γ-globulin (s^0 = 7.38 S, k = 0.00785 L/g, D = 4.8×10^{-7} cm^2/s) subjected to centrifugation at 56,100 rpm. (A) Concentration distributions after the indicated periods (minutes) of centrifugation. (B) Use of Eq. (11) to detect the lack of steady-state attainment by virtue of the non-coincidence of plots for all distributions.


A
Concentration c (g/L)
8
6
4
2
0
25
50
75
100
125
150
175
200
6.2
6.4
6.6
6.8
7.0
7.2
Radial Distance r (cm)
B
[(1 + Kc_p)/(Kc_p) Y
16
8
0
−8
−16
−0.25
−0.15
−0.05
0.05
0.15
0.25
(r^2 − r_b^2) (cm^2)


A
Concentration c (g/L)
8
6
4
2
0
15
30
45
60
75
90
6.2
6.4
6.6
6.8
7.0
Radial Distance r (cm)
B
Concentration c (g/L)
8
6
4
2
0
65
130
195
260
325
390
6.2
6.4
6.6
6.8
7.0
Radial Distance r (cm)


A
15
10
5
0
−5
−10
−15
−0.6
−0.3
0
0.3
0.6
50,000
25,000
[(1 + Kc_p)/(Kc_p)] Y
(r^2 − r_b^2) (cm^2)
B
120
100
80
60
40
20
0
0
1
2
3
4
[(1 + Kc_p)/(Kc_p)] dY/dr^2 (cm^−2)
10^5 ω^2 s^0 (s^−1)




A
Concentration c (g/L)
12
9
6
3
0
25
40
55
70
85
100
5.8
6.0
6.2
6.4
6.6
6.8
7.0
Radial Distance r (cm)
B
(1/cp)Ln[(cp – c)/c] (L/g)
0.30
0.15
0
–0.15
–0.30
25
100
–1.5
–1.0
–0.5
0
0.5
1.0
1.5
(r2 – rb2) (cm2)